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Attorney's Docket No.: 10287-051002 / MGH 1470.2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Michael Detmar et al. Art Unit : 1646
Serial No. : 09/822,682 Examiner : Unknown
Filed : March 30, 2001
Title : THROMBOSPONDIN-2 AND USES THEREOF

BOX MISSING PARTS
Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

In response to the communication dated May 24, 2001 (copy enclosed), applicants submit herewith a Sequence Listing in computer readable form as required by 37 CFR §1.824. In addition, applicants submit an initial Sequence Listing as required under 37 CFR §1.823(a) and a statement under 37 CFR §1.821(f).

Applicants respectfully request entry of the paper copy and computer readable copy of the Sequence Listing filed herewith for the instant application. Furthermore, applicants request entry of the following amendments.

In the specification:

Insert the paper copy of the Sequence Listing following the Oath/Declaration.

Replace the paragraph beginning at page 22, line 21, with the following rewritten paragraph:

--In a preferred embodiment, the TSP-2 polypeptide includes a domain that includes at least one, two or three type 1 repeat(s). Preferably, a type 1 repeat is about 40 to 60, 45 to 55, 47 to 52 amino acids in length, and preferably has about 70%, 80%, 90% or 95% sequence identity with a type 1 repeat of SEQ ID NO:2. For example, a type 1 repeat can be found at about amino acids 382 to 429 of SEQ ID NO:2; about amino acids 438 to 490 of SEQ ID NO:2; about amino

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231

October 23, 2001
Date of Deposit

Maria Keen
Signature

Maria Keen
Typed or Printed Name of Person Signing Certificate

acids 495 to 547 of SEQ ID NO:2. A type 1 repeat of TSP-2 may have one or more of the following activities: (i) may bind the membrane protein CD36; (ii) may promote an inhibitory effect of TSP-2 on endothelial cell migration; (iii) may induce cell apoptosis, e.g., endothelial cell apoptosis; (iv) may have anti-angiogenic activity of TSP-2; or (v) may inhibit unwanted cell proliferation, e.g., a benign or malignant unwanted cell proliferation, e.g., tumour growth. In a preferred embodiment, a TSP-2 peptide is about 4, 5, 6, 7, 8, 10, 15, 20 or 50 amino acids in length and contains a sequence which inhibits endothelial cell migration. For example, the peptide can include a PWAEW sequence (about amino acid residues 386 to 390 of SEQ ID NO:2), or the fragment can include a WSPWAEW sequence (about amino acids 384 to 390 of SEQ ID NO:2), or conservative substitutions of either sequence. Other peptides can include 4, 5 or 6 amino acids from a WSPWAEW (SEQ ID NO:10) sequence or conservative substitutions thereof. In another embodiment, a TSP-2 peptide includes about 5 to 50 amino acids of the type 1 repeat of TSP-2, or about 5 to 50 amino acids of TSP-2 sequence on one or both sides of the type 1 repeat. In a preferred embodiment, the fragment is 4, 5, 6, 7, 10, 15, 20 or 50 amino acids in length and contains a sequence which contains a receptor binding sequence, e.g., a CSVTVG (SEQ ID NO:11) sequence, which binds CD36.--

Replace the paragraph beginning at page 26, line 4, with the following rewritten paragraph:

--In another aspect, the invention features a TSP-2 antibody. The antibody can be a polyclonal or a monoclonal antibody. The antibody can be raised, e.g., against the intact protein or a fragment thereof. In one embodiment, the antibody can bind specifically to a TSP-2 protein or a fragment. In another embodiment, the antibody binds TSP-2 with significantly greater affinity than TSP-1, e.g., 10%, 20% or 50% higher affinity. In a preferred embodiment, the TSP-2 epitope can be a 10, 15, 20 or 30 amino acid peptide of SEQ ID NO:2, e.g., the epitope is a 15-amino acid peptide DKDTTFDLFSISNIN (SEQ ID NO:3). In another preferred embodiment, the epitope can overlap the 15-amino acid peptide epitope of DKDTTFDLFSISNIN (SEQ ID NO:3).--

Replace the paragraph beginning at page 33, line 18, with the following rewritten paragraph:

--Using these methods, full-length TSP-2 has been obtained but the protein yields have been relatively low. Therefore, 293 human embryonic kidney cells were transfected with a different human TSP-2 expression vector. A PCEP4 vector (Invitrogen) was used that was modified as follows: a BM 40 signal peptide sequence was introduced in front of the insertion site of TSP-2, the antibiotic selection gene was replaced with a puromycin gene for fast and efficient antibiotic selection of stably transfected clones, and a total of 8 histidin residues at the C-terminal end have been included to facilitate purification of the recombinant protein. Using this vector, stably transfected 293 cells produce high amounts of the recombinant protein and the use of mammalian cells ensures efficient glycosylation of recombinant TSP-2. Four different recombinant TSP-2 proteins have now been expressed. Construct I expresses selectively the N-terminal procollagen domain of TSP-2 (nucleotides 294-1367), the region with the least homology to TSP-1. Construct 2 expresses, in addition, the type I repeats (nucleotides 294-1883) which contain several biologically active sites including two CSVTCG (SEQ ID NO:11) sequences that mediate binding to the CD36 receptor on endothelial cells. Construct 3 expresses the type I repeats (nucleotides 1383-1883) only. Construct 4 expresses the full-length mature TSP-2 molecule, excluding the signal peptide (nucleotides 294-3755) which is provided in the expression vector. Such recombinant proteins can be used for the generation of monoclonal anti-TSP-2 antibodies, for the establishment of a human TSP-2 ELISA, and for the systemic treatment of experimental tumors.--

Replace the paragraph beginning at page 45, line 14, with the following rewritten paragraph:

--The following synthetic peptides, derived from the amino acid sequence of human TSP-2, were synthesized:

Peptide 1: RESHFRGLLQNVHLVF: procollagen domain, AA 207-222 (SEQ ID NO:6)

Peptide 2: PATCANPSFVEGECCPSC: procollagen domain, AA 366-383 (SEQ ID NO:7)

Peptide 3: FAENETWVVDSCTTCTCKKFKT: procollagen domain, AA 336-357 (SEQ ID NO:8)

Peptide 4: ELIGGPPKTRNMSAC: procollagen domain, AA 315-329 (SEQ ID NO:9)

Peptide 7: WSPWAEW: first type I repeat, AA384-390 (SEQ ID NO:10)--

Replace the paragraph beginning at page 45, line 21, with the following rewritten paragraph:

--HDMEC migration experiments were performed essentially as described above. 1 x 10^5 HDMEC were added to the upper chamber in 300 l of DMEM medium, or in DMEM medium containing 10 Mol/l of the synthetic peptides. All media were supplemented with 10 mg/ml BSA. As shown in Figure 7, in DMEM medium, 212±12 HDMEC/mm^2 migrated to the underside of the inserts (C; column 1). Peptides 1, 2, 3, and 4 did not significantly modify HDMEC migration. Peptide 2 (WSPWAEW; SEQ ID NO:10) inhibited HDMEC migration by 47.6% (111±39 HDMEC/mm^2, column 2). These results reveal an important role of this TSP-2 specific peptide for the anti-angiogenic activity of TSP-2. Importantly, this peptide is distinct from the CSVTCG (SEQ ID NO:11) sequence that has been described to bind to the CD36 receptor on endothelial cells. Dawson et al. (1997) *J. Cell. Biol.* 138:707-717. All assays were performed in quadruplicate.--

REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. I hereby state, as required by 37 C.F.R. §1.821(g), that the enclosed submission includes no new matter.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 10/23/01

Laurie Lawrence

Louis Myers
Reg. No. 35,965
Reg No. 46,593

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

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"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 22, line 21, has been amended as follows:

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